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Rapid Report

Normal levels of DM RNA and myotonin protein kinase in skeletal muscle from adult myotonic dystrophy (DM) patients

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Abstract

A major question about the pathogenesis of myotonic dystrophy (DM) is how the $(CTG)_n$ repeat mutation alters expression of the DM gene and how that is related to disease causation. Most previous studies have found a decrease in DM RNA and protein in patient tissue. In contrast to these reports we find, unexpectedly, that independent of the size of the CTG repeat: (1) there are equal levels of RNA products of mutant and normal alleles, and (2) levels of Mt-PK in skeletal muscle from DM patients is unaltered from normal. These findings are consistent with the recent hypothesis that mutant DM DNA or RNA may cause disease by disrupting the function of other, yet unidentified, genes.

Keywords: Myotonic dystrophy; Muscle; RNA; Protein

Myotonic dystrophy (DM) is an autosomal dominant human genetic disease with multisystem effects including muscle weakness and myotonia [1]. The molecular basis of DM is now known to be the amplification of a trinucleotide repeat $(CTG)_n$ situated in the 3′ non-coding exon of a gene which encodes a putative serine-threonine protein kinase named myotonin protein kinase (Mt-PK) [2–4]. The cellular effects of the $(CTG)_n$ expansion and how they lead to symptomatology are unknown. Since the CTG expansion is not translated into a protein product, previous studies have attempted to correlate the DM phenotype to changes in the levels of DM RNA or protein (DM-PK) in DM affected tissues. Studies on RNA levels in muscle from DM patients have, however, reported conflicting results finding either a decrease in mRNA product of the abnormal allele [5,7,8], or normal allele [6] or in contrast, in congenital DM, a marked increase in mutant mRNA [9] levels. Most studies of the level of Mt-PK in DM skeletal muscle have found a decrease (up to 40%) [8,10].

Since accurate determination of the level of transcription and translation of the DM gene in DM affected tissues is crucial to understanding the pathogenesis of DM, we analyzed the steady state levels of DM RNA products of mutant and normal alleles and levels of Mt-PK in affected skeletal muscle from adult DM patients. Also, as the severity of disease has been directly correlated with the length of the CTG repeat we specifically asked whether there was any correlation between the length of the CTG repeat and the level of expression of the DM gene in affected tissue.

Skeletal muscle biopsy samples from adult DM patients were analyzed as described [9] for the size of the CTG repeat. Three samples from patients of adult DM with repeat size varying from 450 to 900 repeats were selected for further analysis. To distinguish between normal and mutant alleles we took advantage of a Bpm1 exon 10 polymorphism [9]. RNA levels were assessed using a reverse transcriptase-polymerase chain reaction assay. Primers were chosen from different exons (to allow identification of spliced RNA) [11] to reverse transcribe RNA extracted from skeletal muscle samples and to then amplify a region which spans the Bpm1 site. Bpm1 digestion of the amplified DNA allowed separation of normal (178 bp) and mutant (133 bp) alleles. No difference was found in the levels of RNA product between normal and abnormal alleles (Fig. 1). Band intensity comparisons were made between normal and mutant alleles in the same sample with the normal allele (178 bp) serving as an internal control for the mutant allele (133 bp). Interestingly even the patient with a very long repeat (900 repeats) and

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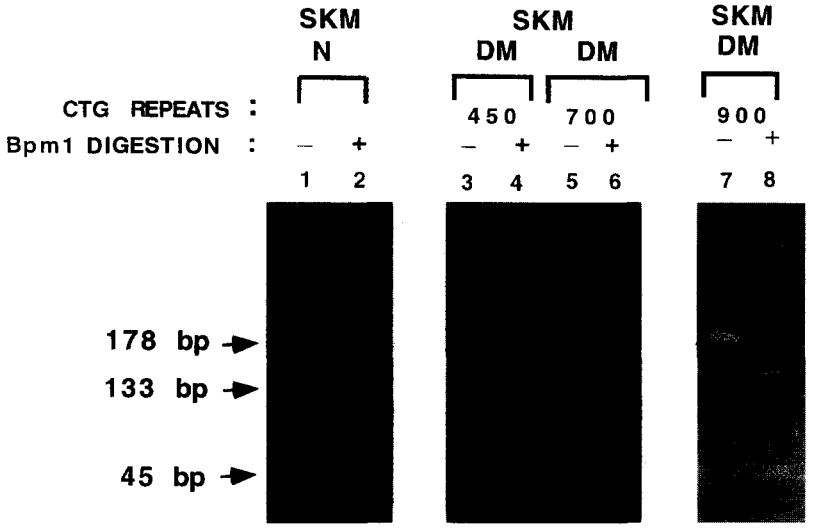

Fig. 1. Reverse transcriptase-polymerase chain reaction assay for steady state levels of spliced DM-mRNA in skeletal muscle from DM patients and a normal individual. PCR printers (5′-CTGTCGGACATTCGGAAGGT-3′ and 5′-CATCCTGTGGGGACACCGAG-3′) were used to amplify a 178 bp fragment spanning the site for the Bpm1 exon polymorphism and an aliquot was digested with Bpm1. Undigested and digested samples have been run on adjacent lanes. Lanes 1,2: Normal skeletal muscle (SKM), age 30 yr; lanes 3,4: DM SKM, age 29 yr, 450 repeats; lanes 5,6: DM SKM, age 22 yr, 700 repeats; lanes 7,8: DM SKM, age 26 yr, 900 repeats. Bpm1 restriction digestion of the abnormal allele yields a 133 bp and 45 bp fragments [11]. Equal levels of RNA products of routant (133 bp) and normal alleles (178 bp) are seen, independent of the size of the CTG repeat on an ethidium bromide stained 3% agarose gel. Negatives of gel pictures were scanned with a LKB densitometer and in each sample the mutant allele was compared to the normal allele.

severe clinical symptoms showed no decrease in the level of transcription from the mutant allele.

Equal transcription from normal and mutant alleles does not exclude the possibility of equivalent decreases or increases in expression from both alleles. We therefore looked next at levels of Mt-PK in skeletal muscle from a normal individual and compared them to levels in the same three DM patients with CTG repeats ranging from 450 to 900. Recently an antibody has been obtained which recognizes the full length Mt-PK [12]. Immunoblotting with this antibody which was raised to a synthetic peptide from the kinase domain of the Mt-PK consistently detected myotonin protein kinase as a single band 72 kilodaltons in size. The 72 kDa DM protein, which represents the full length MtPK isoform [10,12], was present in equal amounts in normal and DM skeletal muscle (Fig. 2).

Most studies of adult DM have reported decreases in the level of processed DM RNA in DM muscle (which is thought to be due to impaired RNA processing) [11,13,14], and also reported decreases in levels of Mt-PK in affected DM skeletal muscle [8,10]. These alterations have been considered important in the pathogenesis of the DM phenotype and consider abnormalities of Mt-PK to be central to the causation of DM. Some of the previous studies to quantitate RNA levels using RT-PCR have not, however, fully considered inefficient action of Taq polymerase and especially reverse transcriptase through the expanded CG rich repeat region. To avoid these inaccuracies we used a DM gene specific primer [11] located 5′ of the repeat region for reverse transcription so that the region analyzed did not include the repeat region. Using this approach we find that the levels of RNA products of normal and mutant alleles are equal. We also find that equal levels of Mt-PK protein can be detected by Western blot analysis in muscle from a normal individual and three DM patients. The CTG repeats in the DM patients we had selected varied from 450 to 900. However no difference in the level of Mt-PK was found. To the best of our knowledge this is the first demonstration that the expression of normal sized Mt-PK, even in severely affected DM muscle, is unchanged from normal. Our findings are in variance with what has been previously reported but inaccuracies in protein estimation can occur due to variations in the extent of muscle fibrosis, tissue preparation and time to freezing and difficulties in accurate quantitation of total protein [15]. Alternatively it can be speculated that the difference between our findings and previous reports may have resulted from the analysis of different subsets of DM patients with varying amounts of Mt-PK in each group. Studies of larger number of DM patients will be needed to clarify this issue.

The finding that skeletal muscle from adult DM patients have normal levels of Mt-PK raises some interesting and important questions about the pathogenesis of this complex disease. Since the DM mutation is located in the 3′-UTR region of the DM gene, alteration of Mt-PK function or toxic alterations of the protein secondary to changes in protein sequence or structure are unlikely. Normal levels of Mt-PK in affected muscle tissue, therefore suggests that the abnormal phenotype may not be caused solely by an alteration of the DM gene product, myotonin protein kinase. The recent findings that knockout mice without any Mt-PK and transgenic animals which overexpress the DM

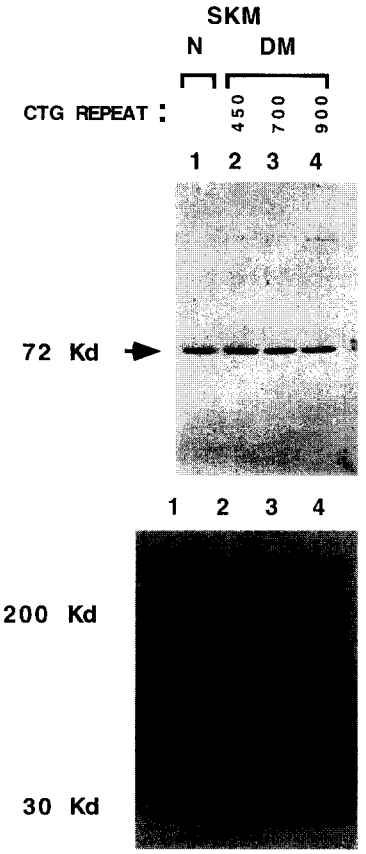

Fig. 2. Immunoblot analysis of DM and normal samples using an antibody to Mt-protein kinase [12]. Top panel, Lane 1, normal skeletal muscle (SKM); lane 2, DM,SKM (450 repeats); lane 3, DM SKM (700 repeats); lane 4, DM SKM (900 repeats). Equal levels of the 72 kDa Mt-PK are seen in all normal and DM samples. Band intensities were quantified with a LKB laser densitometer and the relative value for the amount of DMPK was determined by normalization to the level of actin. Similar results were obtained in three independent experiments. Bottom panel: silver-stained polyacrylamide gel loaded with 15 μg of protein from each of the above samples (lane numbers correspond to samples in top panel).

gene [16] do not exhibit any abnormal phenotype is consistent with this hypothesis. The findings reported here are also consistent with and direct attention to the recently proposed hypothesis [17] that the DM mutation may result in disease by disrupting the function of other, still unidentified, genes.

Alteration of function of another gene may be secondary to altered transcription of an adjacent gene due to alterations in chromatin topology [17]. Recently a large homeodomain encoding gene has been identified in close proximity to the CTG repeat [17] although no abnormalities in the expression of this gene in DM tissue have yet been reported. Alternatively the amplified CTG repeat in mutant DM DNA could titrate out tissue specific DNA binding proteins or mutant DM RNA, although quantitatively normal could affect muscle cell differentiation by sequestering proteins that regulate differentiation [18] or are important in RNA processing. CTG expansion mutation causing such a gain of function, would be consistent with the autosomal dominant inheritance seen in myotonic dystrophy.

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References

[1] Harper, P.S. (1989) Myotonic Dystrophy, W.W. Saunders, London.
[2] Mahadevan, M.S., Tsilfidis, C., Sabourin, L., Shutler, G., Amemiya, C., Jansen, G., Neville, C.E., Narang, M., Barcelo, J. and O'Hoy, K. (1992) Science 255, 1253–1255.
[3] Fu, Y.H., Pizzuti, A., Fenwick, R.G., King, J., Rajnarayan, S., Dunne, P.W., Dubel, J., Nasser, G.A., Ashizawa, T., de Jong, P., Wieringa, B., Korneluk, R., Perryman, M.B., Epstein, H.F. and Caskey, C.T. (1992) Science 255, 1256–1258.
[4] Brook, J.D., McCurrach, M.E., Harley, H.G., Buckler, A.J., Church, D., Aburtani, H., Hunter, K., Stanton, V.P., Thirion, J.P., Hudson, T., Sohn, R., Zemelman, B., Snell, R.G., Rundie, S.A., Crow, S., Davies, J., Shelbourne, P., Buxton, J., Jones, C., Juvonen, V., Johnson, K., Harper, P.S., Shaw, D.J. and Housman, D.E. (1992) Cell 68, 799–808.
[5] Carango, P., Noble, J.E., Marks, J.H. and Funange, V.L. (1993) Genomics 18, 340–348.
[6] Hoffmann-Radvanyi, H., Lavedan, C., Rabes, J., Savoy, D., Duros, C., Johnson, K., and Junien, C. (1993) Hum. Mol. Genet. 2, 1263–1266.
[7] Novelli, G., Gennarelli, M., Fattorini, C., Zelano, G., Dallapiccola, B., Pizzuti, A. and Caskey, C.T. (1993) Biochem. Mol. Biol. Int. 29, 291–297.
[8] Fu, Y.H., Friedman, D.L., Richards, S., Pearlman, J.A., Gibbs, R.A., Pizzuti, A., Ashizawa, T., Perryman, M.B., Scarlato, G., Fenwick, R.G. and Caskey, C.T. (1993) Science 260, 235–238.
[9] Sabourin, L.A., Mahadevan, M.S., Narang, M., Lee, D.S.C., Surh, L.C. and Korneluk, R.G. (1993) Nat. Genet. 4, 233–238.
[10] Maeda, M., Taft, C.S., Bush, E.W., Holder, E., Bailey, W.M., Neville, K., Perryman, M.B. and Bies, R.D. (1995) J. Biol. Chem. 270, 20246–20249.
[11] Krahe, R., Ashizawa, T., Abbruzzese, C., Roeder, E., Carango, P., Giacanelli, M., Funange, V.L. and Siciliano, M.J. (1995) Hum. Mol. Genet. 28, 1–14.
[12] Timchenko, L., Nastainczyk, W., Schneider, T., Patel, B., Hofman, F. and Caskey, C.T. (1995) Proc. Natl. Acad. Sci. USA 92, 5366–5370.
[13] Taneja, K.L., McCurrach, M., Schalling, M., Housman, D. and Singer, R.H. (1995) J. Cell. Biol. 128, 995–1002.
[14] Wang, J., Pegoraro, E., Menegazzo, E., Gennarelli, M., Hoop, R.C., Angelini, C. and Hoffman, E.P. (1995) Hum. Mol. Genet. 4, 599–606.
[15] Roses, A.D. (1994) Science 264, 587.
[16] Johnson, K.J. and Siciliano, M.J. (1995) J. Med. Genet. 32, 662–665.
[17] Boucher, C.A., King, S.K., Carey, N., Krahe, R., Winchester, C.L., Rahman, S., Creavin, T., Meghji, P., Bailey, M.E.S., Chartier, F.L., Brown, S.D. Siciliano, M.J. and Johnson, K.J. (1995) Human Mol. Genet. 4, 1919–1925.
[18] Rastinejad, F. and Blau, H.M. (1993) Cell 72, 903–917.